

Express Mail No.: EV529814455US
International Application No.: PCT/JP2004/017307
International Filing Date: 19 November 2004
Preliminary Amendment Accompanying
Substitute Specification

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) An isolated nucleic acid construct comprising a nucleic acid sequence encoding an LTR-type retrotransposon and a promoter, wherein the LTR-type retrotransposon comprises Intracisternal A particle (IAP)-type retrotransposon or a variant thereof, and wherein the promoter sequence has an activity of 0.1 rlu or greater when determined by a luciferase assay *in vitro*.
2. (Canceled)
3. (Canceled)
4. (Original) A nucleic acid construct according to Claim 1 wherein the retrotransposon encodes a polypeptide having a function.
5. (Currently Amended) A nucleic acid construct according to Claim [1] 4 wherein the function comprises at least one activity selected from the group consisting of transcription activity, reverse transcription activity and integrase activity.
6. (Currently Amended) A nucleic acid construct according to Claim 1 wherein the retrotransposon ~~is an IAP element and~~ has at least one domain selected from the group consisting of LTR, *gag*, *pol* and tRNA binding site, which is conserved against SEQ ID NO: 1.

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7. (Currently Amended) A nucleic acid construct according to Claim 1 wherein the retrotransposon ~~is an IAP element, wherein the nucleic acid thereof~~ has at least one feature selected from the group consisting of a repeat of a sequence of TCCGGGACGAGAAAA in the tRNA binding site immediately located at LTR at the 5' side, and inclusion of two or more repeat sequences TTGCTTCTTGCTCT in the R region.

8. (Original) A nucleic acid construct according to Claim 1 wherein the retrotransposon comprises:

- (a) a polynucleotide having a base sequence set forth in SEQ ID NO: 1 or a fragment sequence thereof;
- (b) a polynucleotide encoding a polypeptide consisting of an amino acid sequence set forth in SEQ ID NO: 2, or 3 and 4, or a fragment thereof;
- (c) a polynucleotide encoding a variant polypeptide consisting of an amino acid sequence set forth in SEQ ID NO: 2, or 3 and 4 with at least one mutation selected from consisting of at least one amino acid substitution, addition and deletion, or a fragment thereof, which possesses a biological activity;
- (d) a polynucleotide being a splice variant or allelic variant of the base sequence set forth in SEQ ID NO: 1, or a fragment thereof;
- (e) a polynucleotide encoding a species homolog of a polypeptide consisting of an amino acid sequence set forth in SEQ ID NO: 2, or 3 and 4, or a fragment thereof;
- (f) a polynucleotide which hybridizes to any of polynucleotides (a) through (e) or the complement thereof under stringent conditions, and encoding a polypeptide having a biological activity; or

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(g) a polynucleotide having at least 70 % identity to any of polynucleotides (a) through (e) or the complement thereof under stringent conditions, and encoding a polypeptide having a biological activity.

9. (Original) A nucleic acid construct according to Claim 1 wherein the nucleic acid sequence encoding the retrotransposon comprises SEQ ID NO: 1.

10. (Canceled)

11. (Canceled)

12. (Currently Amended) A nucleic acid construct according to Claim [+] 1 wherein the promoter sequence is selected from the group consisting of CMV, CA and the variants thereof.

13. (Currently Amended) A nucleic acid construct according to Claim [+] 1 wherein the promoter sequence partially substitutes a portion of 5'LTR of the LTR-type retrotransposon.

14. (Original) A nucleic acid construct according to Claim 13 wherein the promoter sequence substitutes an entirety or portion of the U3 region in the 5'^LTR in the LTR-type retrotransposon.

15. (Currently Amended) A nucleic acid construct according to Claim [+] 1 wherein the promoter sequence is operably linked to the retrotransposon.

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16. (Currently Amended) A nucleic acid construct according to Claim [10] 1 wherein the promoter sequence is located in frame to a transcription initiation site of the retrotransposon at the transcription initiation site of the promoter sequence.

17. (Currently Amended) A nucleic acid construct according to Claim [10] 1 wherein the promoter sequence is a base sequence set forth in any of SEQ ID NO: 5-7, or a portion or variant thereof, and comprises a nucleic acid sequence having promoter activity.

18. (Currently Amended) A nucleic acid construct according to Claim [10] 1 wherein the promoter sequence consists of a nucleic acid sequence set forth in SEQ ID NO: 6 or 7.

19. (Original) A nucleic acid construct according to Claim 1 further comprising a sequence encoding a foreign gene.

20. (Original) A nucleic acid construct according to Claim 19 wherein the sequence encoding the foreign gene is placed in said retrotransposon.

21. (Original) A nucleic acid construct according to Claim 19 wherein the foreign gene renders a host a distinguishable property.

22. (Original) A nucleic acid construct according to Claim 21 wherein the distinguishable property is selected from the group consisting of PCR primer, antibiotic resistance, complement of nutrition, enzymatic activity and fluorescence.

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23. (Original) A nucleic acid construct according to Claim 19, wherein the foreign gene is selected from the group consisting of *neo*, GFP, hyg, puro, zeo, bsr, lacZ, CFP, YFP, RFP, BFP and hrGFP.

24. (Original) A nucleic acid construct according to Claim 19, wherein the foreign gene is composed such that the foreign gene is first expressed only after transcription, reverse transcription and insertion into the genome it is subjected to.

25. (Original) A nucleic acid construct according to Claim 19, wherein the foreign gene comprises an intron sequence.

26. (Original) A nucleic acid construct according to Claim 25, wherein the intron sequence is located in the same transcription direction (forward) with respect to the retrotransposon.

27. (Original) A nucleic acid construct according to Claim 25, wherein the intron sequence is located between a splice donor sequence and a splice acceptor sequence.

28. (Original) A nucleic acid construct according to Claim 1 for use in genomic modification.

29. (Original) A nucleic acid construct according to Claim 11 which is for confirming whether or not the retrotransposon has transposition ability.

30. (Original) A nucleic acid construct according to Claim 19 which is for transposing the foreign gene.

31. (Original) A nucleic acid construct according to Claim 19 which is used for introducing the foreign gene into a host.

32. (Currently Amended) A method for modifying a genome in a cell, comprising the steps of:

- A) providing a nucleic acid construct comprising an LTR-type retrotransposon; and a promoter, wherein the LTR-type retrotransposon comprises Intracisternal A particle (IAP)-type retrotransposon or a variant thereof, and wherein the promoter sequence has an activity of 0.1 rlu or greater when determined by a luciferase assay *in vitro*;
- B) introducing the nucleic acid construct into the cell;
- C) culturing the cell for a predetermined period of time; and
- D) selecting a cell with a genome modified by means of the nucleic acid construct.

33. (Currently Amended) A method according to Claim 32, ~~comprising a promoter having an activity of 0.1 rlu or greater as determined by a luciferase assay *in vitro*,~~ wherein the predetermined period of time is sufficient for transcription, reverse transcription and insertion into the genome.

34. (Original) A method according to Claim 32, wherein the promoter sequence is located in frame to a transcription initiation site of the retrotransposon at the transcription initiation site of the promoter sequence.

35. (Original) A method according to Claim 32, wherein the nucleic acid construct comprises a foreign gene located in an operable manner in the retrotransposon, and the selection is achieved by the expression of the foreign gene.

36. (Currently Amended) A method according to Claim [32] 35, wherein the foreign gene is located in the reverse direction with respect to the transcription direction of the retrotransposon, and comprises a splice donor sequence and splice acceptor sequence, and an intron sequence located *cis*-direction sandwiched therebetween, wherein said predetermined period of time is sufficient for achieving transcription, reverse transcription and insertion into the genome, and wherein the selection is achieved by the expression of the foreign gene.

37. (Original) A method according to Claim 36, wherein the foreign gene encodes an agent selected from the group consisting of a antibiotic resistance gene, nutrient supplement agent, enzyme and fluorophore, and the selection is achieved by the property of the cell expressing the agent.

38. (Canceled)

39. (Canceled)

40. (Original) A method according to Claim 32, wherein the selection is achieved by confirming the transposed sequence by means of ligation mediated PCR.

41. (Original) A method according to Claim 32, wherein the introduction comprises a format selected from the group consisting of transfection, transformation and transduction.

42. (Original) A method according to Claim 32, wherein the introduction is achieved in the presence of at least one substance selected from the group consisting of cationic lipids and polyamine reagents.

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43. (Original) A method according to Claim 32, wherein the cell is of the same species as that of the natural host of the retrotransposon.

44. (Original) A method according to Claim 32, wherein the cell is of the different species as that of the natural host of the retrotransposon.

45. (Original) A method for assaying transposition activity of a retrotransposon, comprising the steps of:

- A) providing a nucleic acid construct comprising a nucleic acid sequence encoding a retrotransposon to be assayed, and a promoter sequence having activity of at least 0.1 rlu as determined by a luciferase assay *in vitro*;
- B) introducing the nucleic acid construct into the cell;
- C) culturing the cell for a predetermined period of time; and
- D) detecting the transposition by means of nucleic acid construct.

46. (Original) A method according to Claim 45, wherein the detection comprises the step of ligation mediated PCR.

47. (Original) A method according to Claim 45, wherein the detection comprises the step of comparing a genomic database and the sequence obtained by the ligation mediated PCR.

48. (Currently Amended) A method for producing the transgenic organism, comprising the steps of:

- A) providing a nucleic acid construct comprising a nucleic acid sequence encoding a LTR-type retrotransposon; and a promoter, wherein the LTR-

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type retrotransposon comprises Intracisternal A particle (IAP)-type retrotransposon or a variant thereof, and wherein the promoter sequence has an activity of 0.1 rlu or greater when determined by a luciferase assay *in vitro*;

- B) introducing the nucleic acid construct into a germ-line cell of a desired biological organism;
- C) selecting a germ-line cell with the genome thereof modified in the germ-line cell; and
- D) regenerating the germ-line cell with the genome thereof modified into a biological organism.

49. (Currently Amended) A kit for modifying the genome of a cell, comprising:

- A) a nucleic acid construct comprising a nucleic acid sequence encoding a LTR-type retrotransposon; and a promoter, wherein the LTR-type retrotransposon comprises Intracisternal A particle (IAP)-type retrotransposon or a variant thereof, and wherein the promoter sequence has an activity of 0.1 rlu or greater when determined by a luciferase assay *in vitro*;
- B) means for introducing the nucleic acid construct into a germ-line cell of a desired biological organism; and
- C) means for selecting a germ-line cell with the genome thereof modified in the germ-line cell.

50. (Original) A kit according to Claim 49, wherein the means for introducing the nucleic acid construct into the cell comprises a transfection reagent.

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51. (Original) A kit according to Claim 48, wherein the transfection reagent is selected from the group consisting of cationic macromolecule, cationic lipid, polyamine reagent, polyimine reagent, and calcium phosphate.

52. (Original) A kit according to Claim 50, wherein the transfection reagent is selected from the group consisting of cationic lipid and polyamine reagent.

53. (Original) A kit according to Claim 49, wherein the means for selection comprises at least one of means for detection corresponding to one selected from the group consisting of a PCR primer, antibiotic resistance, complement of nutrition, enzymatic activity and fluorescence.

54. (Currently Amended) A kit for assaying transposition activity of a retrotransposon, comprising:

A) a nucleic acid construct comprising a nucleic acid sequence encoding a LTR-type retrotransposon, and a promoter having an activity of 0.1 rlu or greater as determined by a luciferase assay *in vitro*, wherein the LTR-type retrotransposon comprises Intracisternal A particle (IAP)-type retrotransposon or a variant thereof;

B) means for introducing the nucleic acid construct into the cell; and

C) means for detecting transposition by the nucleic acid construct.

55. (Original) A kit according to Claim 54, wherein the means for detecting comprises at least one means selected from means for detection of at least one

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of the group consisting of PCR primer, antibiotic resistance, complement of nutrition, enzymatic activity and fluorescence.

56. (Currently Amended) A kit for producing a transgenic organism, comprising:

- A) a nucleic acid construct comprising a nucleic acid sequence encoding an LTR-type retrotransposon; wherein the LTR-type retrotransposon comprises Intracisternal A particle (IAP)-type retrotransposon or a variant thereof;
- B) means for introducing the nucleic acid construct into a germ-line cell of a desired organism;
- C) means for selecting a germ-line cell with the genome thereof modified in the germ-line cell; and
- D) means for regenerating the germ-line with the genome thereof modified into an organism.

57. (Original) A kit according to Claim 56, wherein the means for regenerating the organism comprises an organism as a host.

58. (Original) A promoter comprising a cytomegalovirus enhancer and avian beta-actin promoter, wherein at least one of the cytomegalovirus enhancer and the avian beta-actin promoter comprises a sequence shorter than the native full-length thereof.

59. (Original) A promoter according to Claim 58, wherein the shorter sequence is due to the deletion of a sequence downstream of the transcription initiation site.

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60. (Original) A promoter according to Claim 58, wherein all the sequence down stream of the transcription initiation site is deleted.

61. (Original) A promoter according to Claim 58, wherein a portion of a sequence downstream of the transcription initiation site and the promoter region is deleted.

62. (Original) A promoter according to Claim 58, wherein the cytomegalovirus enhancer comprises a sequence set forth in SEQ ID NO: 36 and a variant thereof.

63. (Original) A promoter according to Claim 58, wherein the avian beta-actin promoter comprises a sequence set forth in SEQ ID NO: 8 or a variant thereof.

64. (Original) A promoter according to Claim 58, comprising the sequence set forth in SEQ ID NO: 6.

65. (Original) A promoter according to Claim 58, comprising the sequence set forth in SEQ ID NO: 7.

66. (Original) A promoter according to Claim 58, consisting of the sequence set forth in SEQ ID NO: 6.

67. (Original) A promoter according to Claim 58, consisting of the sequence set forth in SEQ ID NO: 7.

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68. (Currently Amended) Use of an LTR-type retrotransposon for genomic modification, wherein the LTR-type retrotransposon comprises Intracisternal A particle (IAP)-type retrotransposon or a variant thereof.

69. (Original) Use of a promoter having an activity of 0.1 rlu or greater as determined by a luciferase assay *in vitro*, for modification of a genome.

70. (Original) Use of a promoter having an activity of 0.1 rlu or greater as determined by a luciferase assay *in vitro*, for confirmation of an LTR-type retrotransposon.